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Sequential Sampling of Plasma Cholinesterase in Mallards ('Anas platyrhynchos') as an Indicator of Exposure to Cholinesterase Inhibitors

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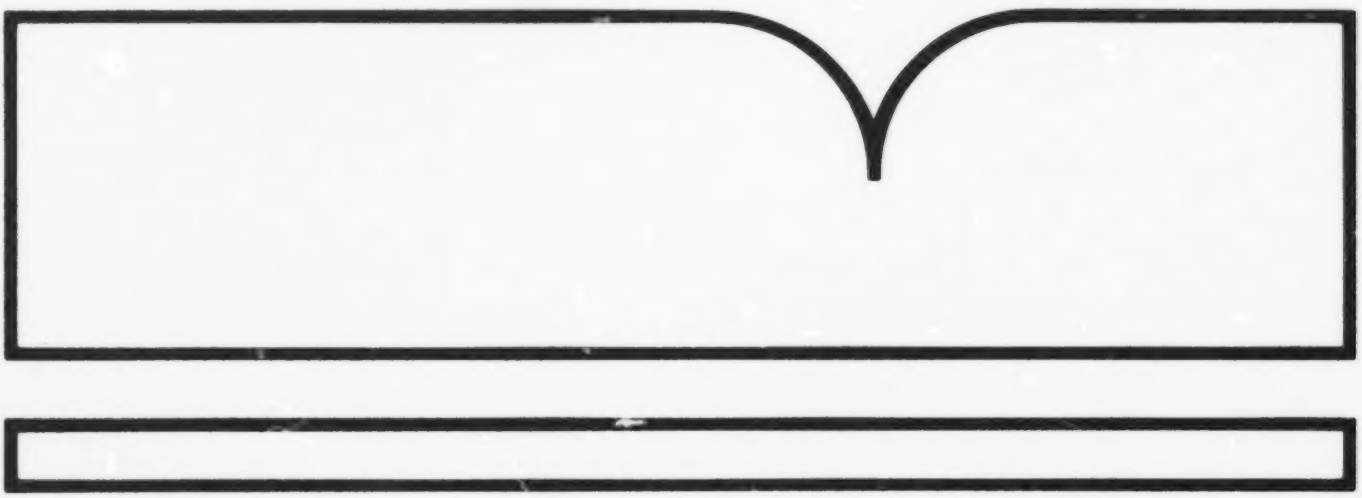

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16. ABSTRACT

The use of sequential measurements of plasma cholinesterase (ChE) activity for monitoring exposure to organophosphorus pesticides was investigated in the mallard (Anas platyrhynchos). At the onset of incubation, birds were assigned to treated (400 ppm methyl parathion in the diet), pair-fed (same daily food allotment on a g/kg/d basis as consumed by the treated bird of the pair), control or nonincubating control groups. Blood samples were collected weekly during egg-laying and every 3 d during incubation periods. Both plasma and brain samples were taken on day 24 of incubation. Plasma ChE was more variable than brain ChE among the birds and there were no significant correlations ($p>0.05$) between plasma and brain samples within treatment groups. Reduced food consumption during incubation did not affect plasma ChE activity ($p=0.77$). Birds that abandoned their nests had significantly increased (paired $t=-2.39$, $d.f.=9$, $p<0.05$) ChE activity at the time of abandonment. There were significant differences ($p<0.01$) in plasma ChE activity between untreated birds, but within-bird variation was 2 and 11 times less than between-bird variation during egg-laying and incubation, respectively. Methyl parathion significantly ($p<0.0001$) reduced plasma ChE activity. Measurement of plasma ChE activity provides a noninvasive, nonlethal technique for assessing the exposure of animals to ChE inhibitors.

Key words: Cholinesterase, Serum chemistry, Repeated measures, Experimental design, Mallard, Methyl parathion

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Environmental Toxicology

SEQUENTIAL SAMPLING OF PLASMA CHOLINESTERASE IN MALLARDS (*ANAS PLATYRHYNCHOS*) AS AN INDICATOR OF EXPOSURE TO CHOLINESTERASE INHIBITORS

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Abstract—The use of sequential measurements of plasma cholinesterase (ChE) activity for monitoring exposure to organophosphorus pesticides was investigated in the mallard (*Anas platyrhynchos*). At the onset of incubation, birds were assigned to treated (400 ppm methyl parathion in the diet), pair-fed (same daily food allotment on a g/kg/d basis as consumed by the treated bird of the pair), control or nonincubating control groups. Blood samples were collected weekly during egg-laying and every 3 d during incubation periods. Both plasma and brain samples were taken on day 24 of incubation. Plasma ChE was more variable than brain ChE among the birds and there were no significant correlations ($p > 0.05$) between plasma and brain samples within treatment groups. Reduced food consumption during incubation did not affect plasma ChE activity ($p = 0.77$). Birds that abandoned their nests had significantly increased (paired $t = -2.39$, $d.f. = 9$, $p < 0.05$) ChE activity at the time of abandonment. There were significant differences ($p < 0.01$) in plasma ChE activity between untreated birds, but within-bird variation was 2 and 11 times less than between-bird variation during egg-laying and incubation, respectively. Methyl parathion significantly ($p < 0.0001$) reduced plasma ChE activity. Measurement of plasma ChE activity provides a noninvasive, nonlethal technique for assessing the exposure of animals to ChE inhibitors.

Keywords—Cholinesterase Serum chemistry Repeated measures Experimental design Mallard Methyl parathion

INTRODUCTION

Measurement of cholinesterase (ChE) activity in animal tissues is one of the few tools, other than residue analysis, available to toxicologists for diagnosing wildlife exposure to organophosphorus and carbamate pesticides [1]. Brain generally has been the tissue of choice for measuring ChE activity, usually by various modifications of the colorimetric method of Ellman et al. [2]. Brain ChE activity is depressed rapidly by ChE inhibitors, decreasing within 2 h and reaching a maximum depression by 24 to 48 h postexposure; activity returns to normal 3 d to four weeks following the exposure period [3–5]. However, brain assay requires killing the animal, thereby eliminating the possibility of following the time course of enzyme inhibition and recovery in a single individual, or of measuring the ChE activity in rare or endangered species. A less invasive technique of monitoring ChE activity is to utilize plasma or serum. This has been done by several investigators in laboratory settings, but it has not become a widely accepted practice because of the large variability among individuals of a given species and the rapidity of return to normal values (within 24 h postexposure) [3–8]. However, those studies used experimental designs such that each animal's blood ChE was measured only once and comparisons were made only between treated and control animals. Blood measurement has the advantage over brain assay of allowing sequential sampling in a single individual. This study examined the feasibility of using sequential blood ChE measurements in single animals and various statistical analytic methods to reduce the confounding influence of individual variability.

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METHODS

This study was part of a larger experiment to assess the effects of methyl parathion (*O,O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate) on nest attentiveness, prolactin and leutinizing hormone levels in mallards (*Anas platyrhynchos*). The entire study design will be described, but results of only the cholinesterase measurements will be presented here.

Six-month-old mallard hens were purchased from Whistling Wings, Inc. (Hanover, IL) and housed in a plastic-skinned greenhouse under ambient light conditions (15-h light:9-h dark to 17-h light:7-h dark in May to July) with an evaporative cooler maintaining daily maximum temperatures of between 19 and 35°C. Nightly minimum temperatures ranged from 4 to 17°C. Birds were kept in 0.8 × 1.6 × 0.8 m wire-bottom, wooden pens. A 0.3 × 0.4 m nest box filled with straw was partially enclosed in one corner of each pen. To provide information on nest attentiveness, the rear end of the box was hinged and the front end suspended by a spring; the weight of the hen in the box triggered a microswitch. An Apple IIE computer recorded the percentage of each 10-min interval that the microswitch was closed. Hens began egg production, completed egg clutches and initiated incubation on their own schedules. Onset of incubation was defined as cessation of egg-laying for 48 h and exhibition of brooding behavior.

Birds were supplied with water and Purina (St. Louis, MO) layer chow ad libitum until the onset of incubation, at which time they were assigned to one of four treatment groups: treated (400 ppm methyl parathion in feed; purity 94.8%); pair-fed (the same daily food allotment on a g/kg/d basis as consumed by the treated bird of the pair); control (unlimited food availability, no chemical in feed); or nonincubating control (birds that laid clutches but did not incubate). The dose of 400 ppm methyl parathion in feed was selected based on previous experiments that demonstrated nest abandonment and changes in reproductive hormones in mallard hens within 8 d following exposure to the chemical (R. S. Bennett et al., manuscript in preparation). Birds were assigned to treatment groups in random blocks; within-block assignments were made in a randomized order.

Prior to onset of incubation, food consumption was measured weekly; during incubation, it was measured every 3 d, but the rations of pair-fed birds were presented daily. Blood samples were drawn weekly by jugular venipuncture until onset of incubation, and every 3 d for 21 d thereafter. Sample volumes were 6 and 4 ml, respectively. Hematocrit (PCV) and total plasma proteins (TP) were measured at each sampling period and did not decrease appreciably during the course of the study (mean ± SE): PCV at start, 38.6 ± 0.8, and at end, 35.2 ± 1.0; TP at start (egg-laying), 7.5 ± 0.4, and at end (incubating), 4.7 ± 0.2. This indicated that the sampling regimen was not a significant hematological stress. Blood for ChE analyses was transferred immediately to 5-ml Vacutainer (Becton-Dickenson, Rutherford, NJ) tubes containing sodium heparin and kept on ice until centrifuged at 3,000 *g* for 10 min at 4°C. Plasma samples were stored at −20°C until assayed for ChE. At the end of the experimental period for each hen, whole brains were excised and homogenized in pH 7.4 Tris buffer at the ratio of 120 mg brain/ml buffer using a Virtis "45" homogenizer (Virtis Co., Gardiner, NY) at 30% power for 15 s. Homogenates were centrifuged for 20 min at 18,800 *g* at 5°C and assayed immediately.

Analysis

Total plasma ChE and brain tissue activities were determined by a modification of the technique of Ellman et al. [2]. Final volumes of reagents in the test were 20 μl 0.075 M acetylthiocholine as substrate (Sigma Chemical Co., St. Louis, MO) and 100 μl 0.1 M 5,5-dithio-bis-(2-nitrobenzoic acid) in pH 8.0 phosphate buffer. The sample volume was 20 μl plasma or 0.4 ml brain supernatant. Assays were conducted at 35°C. Six absorbance readings were taken during a 2-min period beginning 20 s after the start of the reaction. Reference standards (Precilip; Boehringer-Mannheim Diagnostics, Indianapolis, IN) were included with each day's run. All samples were run in duplicate; assays were repeated if duplicate runs differed by more than 10%. The results of the two accepted runs were averaged. Enzyme activity of the plasma was expressed in International Units and represented the amount of enzyme that catalyzed the transformation of 1 μmol substrate/min/L. Brain ChE activity was expressed as μmol substrate hydrolyzed/min/g tissue.

Statistics

Analyses were conducted using the SAS package for personal and mainframe computers [9]. Data were transformed to $\log_{10}$ prior to analysis to normalize the distribution and reduce heterogeneity of variance among birds. The effect of methyl parathion treatment was analyzed using

both a simple and a covariate one-way analysis of variance (ANOVA). Plasma ChE values obtained immediately prior to the start of treatment were used as the covariate and plasma values obtained 3 d after the onset of treatment were used as the response variable. Within- and between-bird variance was determined using repeated-measures ANOVAs for control and treated groups separately, following procedures described by Winer [10] such that PEN (between-bird variation) was the main effect and the ERROR term was split into a TIME PERIOD effect (within-bird variation) and ERROR term. Because treatment groups were analyzed separately, this reduced the repeated measures design to a more simple two-way ANOVA. Means of plasma ChE values of treated, control and paired birds during egg-laying and incubation periods were compared using Tukey's studentized range test. Correlations between brain and plasma ChE activities within treatment groups were examined using regression analysis.

RESULTS

Plasma ChE was more variable than brain ChE when single plasma samples per bird were considered. Comparisons within treatment groups of plasma and brain ChE from samples collected at death showed that the coefficients of variation for plasma were 1.3 to 4.7 times greater than those for brain (Table 1). There were no significant correlations ($p > 0.05$) between plasma and brain samples within treatment groups.

Nineteen of forty-seven hens had one or two ChE values during egg-laying that were at least twice as high as any of their other values. An extreme example of this occurred in one hen whose average (±SE) ChE value of 9 of 10 preincubation samples was 1,400 ± 85 IU/L (untransformed data), as compared with one sample with a value of 4,790 IU/L. Since no procedural or biological explanation for these high values could be found, none were dropped from the data set. Similarly high ChE values were not observed during incubation. Reduced food consumption during incubation in the pair-fed group did not affect plasma ChE activity as compared with that in control birds ($p = 0.77$). Therefore, data obtained during incubation from birds of both groups ($N = 22$) were combined to evaluate interbird variability before and during incubation. Postabandonment ChE values were excluded from the analysis because birds that abandoned their nests had a significant increase (paired $t = -2.37$, $d.f. = 9$, $p < 0.05$) in ChE activity at the time of abandonment. No temporal trends were observed in ChE activity during incubation.

There were significant differences ($p < 0.001$) in plasma ChE activity among all nontreated birds (control and pair-fed, $N = 22$) before and during incubation (Table 2). Examination of the ANOVA tables (Table 3) shows that within-bird variation (ERROR mean square) was two times less than between-bird variation (PEN mean square) during egg-laying, and 11 times less during the incubation period. For treated incubating birds, between-bird variability was two times greater than within-bird variability (Table 3).

One-way ANOVA of plasma ChE activity among the three treatment groups during incubation ($N = 34$) using ChE values from blood samples obtained immediately prior to treatment (i.e., at the onset of incubation) as a covariate and the first sample obtained after onset of treatment (at 3 d) as the response variable, indicated that the treated group had significantly lower values ($p < 0.0001$) than the others. This analysis was repeated as a simple one-way ANOVA (no covariate); the results were unchanged due to the highly significant effects of the methyl parathion treatment.

Table 1. Mean (± SD) and coefficient of variation (%) for mallard brain and plasma cholinesterase (ChE) activity at death in four treatment groups[a]

Treatment group	N	Brain ChE[b]	Plasma ChE[c]
Nonincubating control	11	4.65 ± 0.18 3.8%	1,182 ± 212 17.9%
Control	11	4.68 ± 0.32 6.8%	1,093 ± 301 27.5%
Treated	10	2.43 ± 0.40 16.4%	220 ± 45 20.5%
Pair-fed	10	4.74 ± 0.36 7.5%	1,043 ± 214 20.5%

[a]Only birds for which both brain and plasma samples were collected on the same day were included in the analysis.
[b]Activity expressed as μmol hydrolyzed/min/g tissue.
[c]Activity expressed as IU/L.

DISCUSSION

The data from this experiment clearly show considerable variability in plasma ChE activity levels among individual mallards. However, the data also indicate that values for each individual remained relatively constant, unless influenced by hormonal perturbations such as prolactin decrease following cessation of incubation. This informa-

Table 2. Mean (± SE) of the $\log_{10}$ plasma cholinesterase activity (IU/L) in egg-laying and incubating mallards

Bird no.	Treatment[a]	Mean (SE)[b]	Bird no.	Treatment[a]	Mean (SE)[b]
Egg-laying			Incubating		
4	PF	2.789 (0.071) A	29	CON	2.830 (0.023) A
3	CON	2.847 (0.056) A,B	3	CON	2.881 (0.016) A,B
13	PF	2.976 (0.060) A,B,C	25	PF	2.895 (0.020) A,B,C
33	CON	3.028 (0.046) A,B,C	13	PF	2.913 (0.013) A,B,C,D
18	CON	3.031 (0.125) A,B,C	4	PF	2.943 (0.011) A,B,C,D,E,F,G
42	PF	3.047 (0.046) A,B,C	30	CON	2.946 (0.015) B,C,D,E
8	PF	3.055 (0.064) A,B,C	33	CON	2.950 (0.011) B,C,D,E
25	PF	3.055 (0.078) A,B,C	21	PF	2.951 (0.027) B,C,D,E,F
16	PF	3.066 (0.061) A,B,C	8	PF	2.957 (0.011) B,C,D,E,F
11	CON	3.093 (0.060) A,B,C	5	PF	2.990 (0.026) B,C,D,E,F,G
43	CON	3.106 (0.114) A,B,C	16	PF	2.990 (0.013) B,C,D,E,F,G
5	PF	3.107 (0.059) A,B,C	24	CON	3.004 (0.012) C,D,E,F,G
46	PF	3.125 (0.166) A,B,C	46	PF	3.006 (0.047) D,E,F,G
38	CON	3.135 (0.089) A,B,C	18	CON	3.017 (0.025) D,E,F,G
17	CON	3.152 (0.050) B,C	38	CON	3.020 (0.017) D,E,F,G
21	PF	3.159 (0.069) A,B,C	23	CON	3.023 (0.011) D,E,F,G
6	PF	3.172 (0.061) A,B,C	42	PF	3.024 (0.123) B,E,F,G,H
30	CON	3.191 (0.157) A,B,C	26	CON	3.031 (0.022) E,F,G,H
29	CON	3.197 (0.148) B,C	17	CON	3.047 (0.016) D,F,G,H
26	CON	3.210 (0.071) B,C	11	CON	3.058 (0.021) F,G,H
23	CON	3.259 (0.113) C	43	CON	3.069 (0.014) G,H
24	CON	3.266 (0.075) C	6	PF	3.136 (0.030) H

[a]CON, control; PF, pair-fed (same daily food allotment on a g/kg/d basis as consumed by the experimental bird of the pair, during egg-laying only).
[b]Means in a column having a common letter are not significantly different ($p > 0.05$).

Table 3. Analysis of variance tables showing relative variability of plasma cholinesterase between birds (PEN mean square) and within birds (ERROR mean square) for untreated mallards (control and pair-fed) before incubation, and during incubation for control and pair-fed and treated birds

Source	*d.f.*	Sum of squares	Mean square	*F*	PR > *F*
Untreated (control and pair-fed)					
Between birds	21	1.485	0.0707	2.65	0.0009
Within birds					
Time periods[a]	8	0.783	0.0978	3.66	0.0010
Residual	86	2.299	0.0267		
Corrected total	115	4.567			
Incubation (control and pair-fed)					
Between birds	21	0.732	0.0349	11.28	0.0001
Within birds					
Time periods[a]	7	0.035	0.0050	1.62	0.1379
Residual	112	0.346	0.0031		
Corrected total	140	1.113			
Incubation (treated)					
Between birds	11	0.319	0.0290	3.02	0.0035
Within birds					
Time periods[a]	7	2.750	0.3929	40.87	0.0001
Residual	52	0.500	0.0096		
Corrected total	70	3.569			

[a]Time period = 3-d blood sampling interval.

tion suggests the usefulness of repeated-measures experimental designs when pretreatment values are used as covariates to reduce between-bird variation and to allow for the expression of treatment effects. In the current study, treatment with 400 ppm methyl parathion in the food during incubation had such an overwhelming depressant effect on plasma ChE that between-bird variability was greatly reduced and treatment effects were highly significant whether or not a covariate analysis was used. Other investigators have found similar degrees of depression of plasma ChE by high doses of ChE inhibitors, allowing them to make use of the information without using a covariate or repeated measures approach. Dieter [11] and Dieter and Ludke [12] demonstrated a linear relationship between dose of ChE inhibitor and plasma ChE. However, subtle decreases in plasma ChE that would occur at lower doses of ChE inhibitors probably would not be detected without the use of covariate analysis. Brust et al. [13] used a repeated-measures design to demonstrate that Leghorn chickens (*Gallus gallus*) developed a tolerance to the ingestion of chlorpyrifos (*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate), with an initial decrease in blood ChE followed by a return to control values within 5 weeks after exposure.

Such repeated-measures testing of plasma ChE apparently can be useful in field situations as well as in laboratory experiments. Finley [14] successfully used this technique to document exposure, and subsequent recovery, of blue grouse (*Dendragapus obscurus*) to phosphamidon (2-chloro-2-diethylcarbamoyl-1-methylvinyl dimethyl phosphate) during a field trial in Montana. Use of repeated plasma ChE activity measures could increase the sophistication of chemical hazard evaluation in field trials. In studies using radiotagged animals, blood samples could be obtained at the time of tagging and at subsequent recaptures, enabling researchers to correlate ChE activity with other sublethal endpoints. If pretreatment values were not available, repeated measures still would enhance the power of an ANOVA, especially if a split-plot design [9] were used.

Hormonal variation may contribute to some of the observed within-bird variability in plasma ChE activity in animals not exposed to ChE inhibitors. Estrogen caused an increase in rat (*Rattus* spp.) serum ChE levels [15] and pregnancy and oral contraceptives significantly depressed human serum ChE values (A. Fairbrother et al., manuscript in preparation). In the present experiment, several mallards exhibited significant increases in plasma ChE activity. One increase occurred within 1 to 3 d following nest abandonment and may have been related to falling prolactin levels; in this case, ChE remained elevated. The other was a short-term increase that occurred seemingly at random during the egg-laying period in 5 of 47 hens. In these hens, ChE activity increased to two to three times the baseline values. We hypothesized that there might be a connection between ChE and luteinizing hormone levels, with a peak in plasma ChE activity at or near oviposition. However, using a limited, separate sample of normal mallard hens ($N = 6$), we were unable to find any increase in plasma ChE levels 0 to 2 h after oviposition as compared with values obtained 4 to 6 h later. While the spikes observed in the present experiment remain unexplained, there is no evidence that they were outside the normal variation within ovulatory birds.

Brain and plasma ChE activities of intoxicated birds do not appear to correlate well. In the present study, there was no such correlation. Smith et al. [16] measured both brain and plasma ChE in black-crowned night herons (*Nycticorax nycticorax*) following exposure to fenthion (*O,O*-dimethyl *O*-[3-methyl-4-(methylthio)phenyl] phosphorothioate) and found that brain ChE was not depressed but plasma ChE was. Ludke et al. [5] concluded that plasma ChE does not necessarily respond in the same manner as brain ChE to organophosphate exposure. The lack of a correlation between brain and plasma ChE activities is not surprising. Brain ChE activity is primarily acetylcholinesterase ("true" ChE), although recent studies have shown pseudocholinesterase activity in neuronal tissue as well [17]. Plasma ChE contains varying ratios of acetyl- and butyryl("pseudo")cholinesterase, depending on the species [17–20]. Additionally, both types of ChE are composed of several different molecular subspecies [17,20]. All of these esterases can act to varying degrees on both the acetylthiocholine and butyrylthiocholine substrates used in the colorimetric assay [21]. Consequently, plasma ChE assays reflect the activities of more types of esterases than do brain ChE measurements.

There is no information in the literature about the relative responsiveness of the different esterases to ChE-inhibiting compounds, although it is possible to assay samples for activity of both of the two main esterase classes through competitive inhibition techniques [21]. It has been suggested that the circulating plasma esterases can help buffer the

impact of ChE inhibitors on the central nervous system (CNS) by binding to chemical first, thus decreasing the amounts that subsequently enter the CNS and bind to neuronal ChE [20,22]. This could explain both why plasma ChE has been observed to decrease more rapidly than brain ChE postexposure [3–8] and the lack of direct correlation between the two. If the primary objective of the ChE activity measurement is to document exposure of an animal to a ChE inhibitor, it may not be necessary to directly correlate plasma activity with effects in brain or CNS ChE, particularly since little is known about the significance of effects of sublethal changes in levels of CNS ChE on behavior.

In summary, measurement of plasma ChE activity provides a noninvasive, nonlethal mechanism for assessing the exposure of animals to ChE inhibitors. The present study demonstrates that the variation of plasma ChE levels within a single individual is substantially less than the variation among individuals. Additionally, the use of statistical tests such as covariate analysis can potentially further reduce the confounding influence of normal individual variation, thereby enhancing the usefulness of blood ChE measurements. This study used a relatively high dose of ChE inhibitor; further investigations using more typical field application rates would more fully describe the usefulness of sequential plasma ChE measurements in monitoring pesticide exposure and effects.

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REFERENCES

1. **Hill, E.F.** and **W.J. Fleming.** 1982. Anticholinesterase poisoning of birds: Field monitoring and diagnosis of acute poisoning. *Environ. Toxicol. Chem.* **1**:27–38.
2. **Ellman, G.L., K.D. Courtney, V. Andres** and **R.M. Featherstone.** 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **7**:88–95.
3. **Fleming, W.J.** and **S.P. Bradbury.** 1981. Recovery of cholinesterase activity in mallard ducklings administered organophosphorus pesticides. *J. Toxicol. Environ. Health* **8**:885–897.
4. **Fleming, W.J.** and **C.E. Grue.** 1981. Recovery of cholinesterase activity in five avian species exposed to dicrotophos, an organophosphorus pesticide. *Pestic. Biochem. Physiol.* **16**:129–135.
5. **Ludke, J.L., E.F. Hill** and **M.P. Dieter.** 1975. Cholinesterase (ChE) response and related mortality among birds fed ChE inhibitors. *Arch. Environ. Contam. Toxicol.* **3**:1–21.
6. **Fleming, W.J.** 1981. Recovery of brain and plasma cholinesterase activities in ducklings exposed to organophosphorus pesticides. *Arch. Environ. Contam. Toxicol.* **10**:215–229.
7. **Westlake, G.E., P.J. Bunyan, A.D. Martin, P.I. Stanley** and **L.C. Steed.** 1981. Organophosphate poisoning: Effects of selected organophosphate pesticides on plasma enzymes and brain esterases of Japanese quail (*Coturnix coturnix japonica*). *J. Agric. Food Chem.* **29**:772–778.
8. **Westlake, G.E., P.J. Bunyan, A.D. Martin, P.I. Stanley** and **L.C. Steed.** 1981. Carbamate poisoning: Effects of selected carbamate pesticides on plasma enzymes and brain esterases of Japanese quail (*Coturnix coturnix japonica*). *J. Agric. Food Chem.* **29**: 779–785.
9. **SAS Institute, Inc.** 1985. *SAS/STAT Guide for Personal Computers*, 6th ed. Cary, NC.
10. **Winer, B.J.** 1971. *Statistical Principles in Experimental Design.* McGraw-Hill, New York, NY, pp. 261–283.
11. **Dieter, M.P.** 1974. Plasma enzyme activities in *Coturnix* quail fed graded doses of DDE, polychlorinated biphenyl, malathion, and mercuric chloride. *Toxicol. Appl. Pharmacol.* **27**:86–98.
12. **Dieter, M.P.** and **J.L. Ludke.** 1975. Studies on combined effects of organophosphates and heavy metals in birds: I. Plasma and brain cholinesterase in *Coturnix* quail fed methyl mercury and orally dosed with parathion. *Bull. Environ. Contam. Toxicol.* **13**:257–262.
13. **Brust, R.A., S. Miyazaki** and **G.C. Hodgson.** 1971. Effect of Dursban in the drinking water of chicks. *J. Econ. Entomol.* **64**:1179–1183.
14. **Finley, R.B.** 1965. Adverse effects on birds of phosphamidon applied to a Montana forest. *J. Wildl. Manage.* **29**:580–591.
15. **Illsley, N.P.** and **C.A. LaMartinier.** 1981. Endocrine regulation of rat serum cholinesterase activity. *Endocrinology* **108**:1737–1743.
16. **Smith, G.J., J.W. Spann** and **E.F. Hill.** 1986. Cholinesterase activity in black-crowned night-herons exposed to fenthion-treated water. *Arch. Environ. Contam. Toxicol.* **15**:83–86.
17. **Brimijoin, S.** 1983. Molecular forms of acetylcholinesterase in brain, nerve, and muscle: Nature, localization, and dynamics. *Prog. Neurobiol* **21**:291–322.
18. **Bunyan, P.J., D.M. Jennings** and **A. Taylor.** 1968. Organophosphorus poisoning: Some properties of avian esterases. *J. Agric. Food Chem.* **16**:326–331.
19. **Unakami, S., S. Suzuki, E. Nakanishi, K. Ichinohe, M. Hirata** and **Y. Tanimoto.** 1987. Comparative studies on multiple forms of serum cholinesterase in various species. *Exp. Anim.* **36**:199–204.
20. **Wills, J.H.** 1972. The measurement and significance of changes in the cholinesterase activities of erythrocytes and plasma in man and animals. *C.R.C. Crit. Rev. Toxicol.* **1**:153–202.
21. **Aldridge, W.N.** 1953. The differentiation of true and pseudo cholinesterase by organo-phosphorus compounds. *Biochem. J.* **53**:62–67.
22. **Russell, R.W.** and **D.H. Overstreet.** 1987. Mechanisms underlying sensitivity to organophosphorus anticholinesterase compounds. *Prog. Neurobiol.* **28**: 97–129.